Cumhuriyet Sci. J., 45(3) (2024) 490-495
DOI: https://doi.org/10.17776/csj.1455893

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Supramolecular Solvent Liquid Phase Microextraction of Voriconazole İn Pharmaceutical and Environmental Samples With High Performance Liquid Chromatography Detection

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Research Article

History
Received: 20/03/2024
Accepted: 14/08/2024

ABSTRACT

In this study, a new analytical method based on the supramolecular solvent liquid phase microextraction (Ss-LPME) and HPLC was developed for the analysis of voriconazole as an active drug in the class of antifungals with a wide spectrum of action at trace levels. Nano/micro sized supramolecular phase formed in 1-decanol, tetrahydrofuran and aqueous environment was used as extraction agent for separation and preconcentration of trace level of voriconazole. For the optimization of the Ss-LPME method, important analytical parameters such as the effect of sample solution pH, volume of 1-decanol, amount of THF, ultrasounic irridation time, centrifugation time and sample solution volume on the extraction efficiency were evaluated. Optimal conditions of the Ss-LPME; pH: 8, 1-decanol volume: 200 µL, THF volume: 300 µL, ultrasounic irridation time:10 min and centrifugation time: 8 min. For the developed Ss-LPME/HPLC procedure, the limit of detection (LOD), limit of quantification (LOQ) and enhancement factor (EF) were found 2.7 µg·L^{-1}, 8.8 µg·L^{-1} and 36, respectively. The Ss-LPME/HPLC procedure was applied to waste water, lake water and drug samples. The fact that recovery values ranging from 100% to 108.5% were obtained for these real samples proved that this method can be used successfully in the matrix environments studied.

Keywords: Voriconazole, anaylsis, Supramolecular solvent liquid phase microextraction, Preconcentration, High performance liquid chromatography.

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Introduction

Voriconazole is a triazole in the antifungal class. Voriconazole prevents invasive aspergillosis and Scedosporium apiospermum and Fusarium spp. FDA approval was given in 2002 for patients who did not respond to or were resistant to other treatments. Voriconazole, a new generation triazole antifungal, is used for the treatment of life-threatening fungal infections in immunocompromised patients. Use of voriconazole has increased since the drug was launched in 2002 [1-3]. Due to excessive use of voriconazole is increasing day by day, It is important to determine the correct concentration of the voriconazole active ingredient in drug formulations and environmental samples due to its negative effects on humans and especially ecosystems as a result of its spread into the environment [4,5].

There are several analytical instruments to analyze voriconazole and other pharmaceuticals in different matrix mediums. They are mainly high performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS/MS), high resolution mass spectrometry including time of flight (TOF), raman spectroscopy and electrochemical sensors. However, the disruptive effects of foreign species on the analysis in the matrix environment where the voriconazole drug active ingredient is located and the fact that the voriconazole concentration that may be present at ultra-trace levels is below the detection limit of these devices are the main problems that limit accurate and sensitive analysis [6-8]. Selective extraction and enrichment of trace levels of voriconazole molecules from the matrix medium into a purer analysis medium before analysis is the most effective way to solve this problem [9,10].

Liquid phase microextraction techniques (LPME), in which solvent and chemical consumption is minimal during the sample preparation process, the sample preparation process is very fast, and basic laboratory equipment is used, meet most of the requirements of green analytical methods developed in recent years [11,12]. By using deep eutectic solvents, ionic liquids, supramolecular solvents and switchable solvents, which are in the green solvent class, as extraction agents in LPME methods, these methods are closer to the classification of green analytical methods [13, 14]. Supramolecular solvents, which belong to the class of green solvents, are nano/micro structured liquids formed as a result of mixing compounds with long carbon chains containing hydroxyl (OH) and carboxylic acid (COOH) groups in an aqueous environment with compounds such as tetrahydrofuran

and tetrabutyl ammonium [16, 17]. Supramolecular solvents, which consist of amphiphiles dispersed in a phase, interact strongly with analytes because they both have polar-nonpolar functional groups and contain hydrogen bonds in their structures [16, 17].

In this study, a new liquid phase microextraction technique based on the formation of supramolecular solvent was developed by combining the advantages of LPME and supramolecular solvents to separate and enrich the trace level of voriconazole drug substance from the matrix medium before its determination by HPLC. The Ss-LPME/HPLC procedure was applied to waste water, lake water and drug samples with high recovery values ranging from 100% to 108,5%.

Experimental

Instruments and Chemicals

The determination of the analyte in the samples, which were pre-separated and enriched by the liquid-liquid microextraction method, was carried out with an Agilent brand HPLC device. Optimum working conditions for HPLC analysis of voriconazole: Mobile phase: 40 mM pH 6 phosphate buffer and acetonitrile mixture at (70:30) flow rate of mobile phase: 0.7 mL·min^{-1}, retention time: 4.0 min, column: ACE S C8, Detector: DAD, 254 nm wavelength. Bandelin brand DT-255 model ultrasonic bath was used during the preparation of the mobile phase and microextraction. Sartorius brand PT-10 model pH meter was used to measure the pH of the solutions. In the study, Hettich Rotofix 32 brand centrifuge device was used to separate the aqueous phase and organic extraction phase after microextraction. VWR international brand vortex device was used in vortex mixing processes. All of these chemicals were used in analytical purity. Stock and intermediate stock solutions used for the determination of voriconazole by the liquid phase microextraction method were prepared at appropriate concentrations using deionized water. Voriconazole stock solution at 100 mg·L^{-1} concentration was prepared by weighing 100 mg of pure voriconazole and completing the volume to 1000 mL with pure water. Acetone, 1-propanol and acetonitrile were obtained from Merck. 1-decanol, ethanol and THF were obtained from Sigma-Aldrich.

Liquid Phase Microextraction-HPLC Procedure

An appropriate volume of the stock solution containing 100 mg·L^{-1} of voriconazole was taken into a 50 mL centrifuge tube and the volume was completed to 10 mL with pure water. By adding 2.0 mL of pH 8 buffer to this solution, the pH was fixed at 8. Then, 200 µL 1-decanol and 300 µL of THF were added into the solution. The resulting mixture was kept in an ultrasonic bath for 10 minutes. In this way, nano and micro sized supramolecular extraction solvent drops were formed [16, 17]. The mixture was centrifuged for 8 minutes for extraction and separation of the aqueous phase. The resulting analyte phase was taken with a syringe and methanol was added and the final volume was completed to 1.0 mL. The obtained sample was vialed with appropriate process and measured on the HPLC device. The same procedures were applied to blank samples.

Real Sample Applications

The developed method was applied to industrial wastewater sample, lake water sample and solid form pharmaceutical sample. The water samples taken were filtered through filters with a pore diameter of 0.22 microns before use. 100 mg of drug sample was dissolved in 100 mL of pure water and the developed method was applied to certain volumes taken from the resulting aqueous phase [18]. In addition, the accuracy of the method was checked by applying the developed method by adding voriconazole, whose concentration is precisely known, to these samples.

Results and Discussion

In order to separate and enrich voriconazole with the liquid phase microextraction method, optimum conditions were determined by examining the important analytical parameters on the extraction efficiency including sample solution pH, volume of 1-decanol, volume complementary solvent type, effect of ultrasonication time, model solution volume and centrifuge time. Real sample analyzes and the accuracy of the method were tested under the optimal experimental conditions.

Effect of Sample Solution pH on Extraction Efficiency of Ss-LPME

To determine the optimum sample solution pH for the separation/enrichment of voriconazole by the Ss-LPME, model solutions with pH ranging from 2-10 were prepared. Each stage was run in 3 repetitions in 10 mL model solutions. It was observed that the extraction efficiency increased from pH 2 to higher pH values, reaching a maximum at pH 8, but after pH 8, the extraction efficiency was decreased. The reason for this could be that voriconazole would be in more molecular forms when the pH was increased from 2 to 8, and yet they would be easily degraded in acidic conditions. However, the extraction efficiency slightly go down when the pH of solution was increased from 8 to 10, indicating that the liquid phase microextraction system reached saturation at pH 8. For this reason, the optimum working pH was determined as 8.0 (Figure 1).

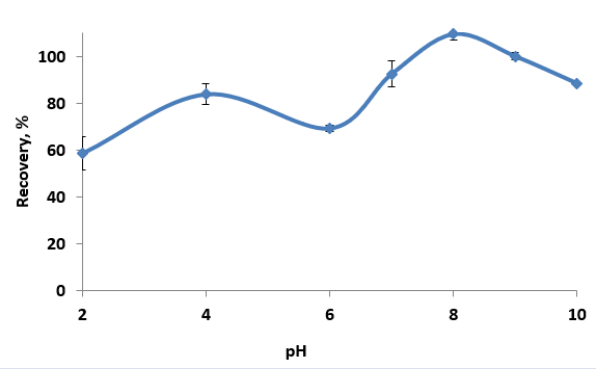

Figure 1. Effect of sample solution pH on the extraction efficiency of voriconazole (N=3).

Effect of 1-Decanol Volume on Extraction Efficiency of Ss-LPME

1-Decanol volumes varying between 50, 100, 150, 200 and 250 µL were added to the model solutions prepared at pH 8.0, and the effect of 1-Decanol volume on extraction efficiency was screened. In the method developed for the determination of voriconazole by liquid phase microextraction, as the volume of 1-decanol added to the mixture increased, the amount of analyte passing into the solvent phase increased. As seen in Figure 2, the maximum recovery values were obtained at maximum 200 µL and 250 µL of 1-Decanol volumes. When 200 µL 1-Decanol was used, the maximum analyte passed into the solvent phase and remained constant after this value. Therefore, 200 µL of 1-Decanol was recorded as the optimum value for the separation and preconcentration of voriconazole by Ss-LPME.

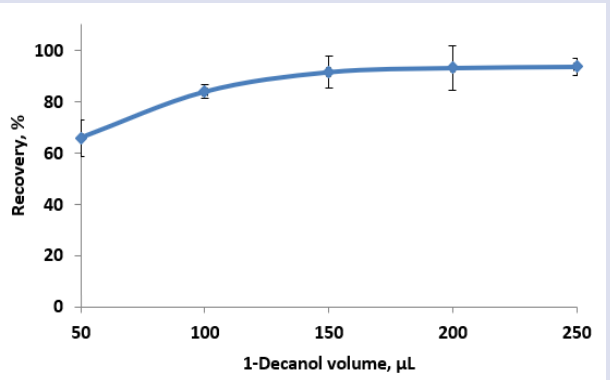

Figure 2. Effect of 1-Decanol volume on the extraction efficiency of voriconazole (N=3).

Effect of THF Volume on Extraction Efficiency of Ss-LPME

In order to determine the THF volume on extraction efficiency, 100, 200, 300, 400 and 500 µL THF volumes were added to test tubes containing voriconazole model solution with pH 8.0 and 200 µL 1-decanol. Then, the samples were centrifuged after being kept in an ultrasonic bath. The resulting analyte phase was taken, its volume was measured, and methanol was added to make up to 1.0 mL, and analyzes were carried out by HPLC. The optimum THF volume for the determination of voriconazole by liquid phase microextraction was determined as 300 µL.

Effect of Volume Complementary Solvent Type

After liquid phase microextraction of voriconazole, the extractant phase was completed to 1.0 milliliter with various organic solvents to obtain a homogeneous phase that could be injected into HPLC. For this purpose, experimental studies were carried out with HPLC grade solvents including isopropanol, ethanol, acetonitrile, methanol and 50% methanol-50% ethanol mixtures. As shown in Figure 3, the highest peak area was obtained when methanol was used as the solvent. Methanol was observed as the ideal solution for the injection of extraction phase for the determination of voriconazole by Ss-LPME. To determine the optimum methanol volume, final volumes ranging from 0.5 to 3.5 mL were injected into HPLC. The highest peak area was obtained when the extractant phase was completed to 1.0 mL with methanol after extraction. In the determination of voriconazole by Ss-LPME, the ideal volume complementary solvent was selected as 1.0 mL methanol.

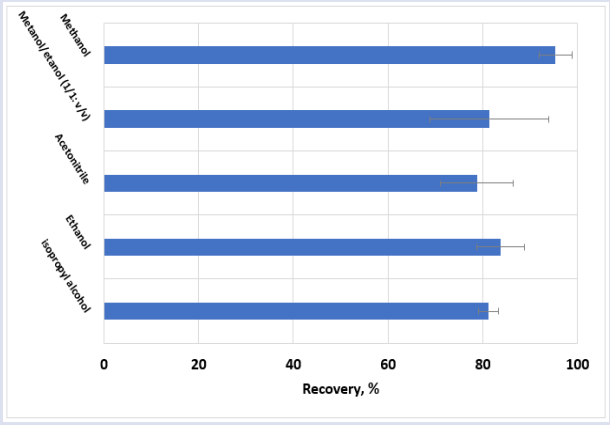

Figure 3. Effect of complementary solvent type on the recovery of voriconazole (N=3).

Effect of ultrasonic irridation time on extraction efficiency of Ss-LPME

Ultrasonic irridation effect was used to obtain nano and micro-sized supramolecular extraction droplets [16, 17]. To determine the optimum ultrasonic vibration time, the tubes prepared by adding 2..0 mL pH 8 buffer, 200 µL decanol and 300 µL THF were exposed to ultrasonic vibration for 2, 4, 6, 8 and 10 minutes, respectively. At each stage, each sample was kept in an ultrasonic bath for the specified periods of time and centrifuged for 8 minutes. The resulting analyte phase was taken, its volume was measured, and methanol was added to make it up to 1.0 mL, and then it was given to the HPLC. As seen in Figure 4, the highest recovery value was obtained at 10 minutes of ultrasonic irridation time. The optimum ultrasonic bath time for the determination of voriconazole by the liquid phase microextraction method was recorded as 10 minutes.

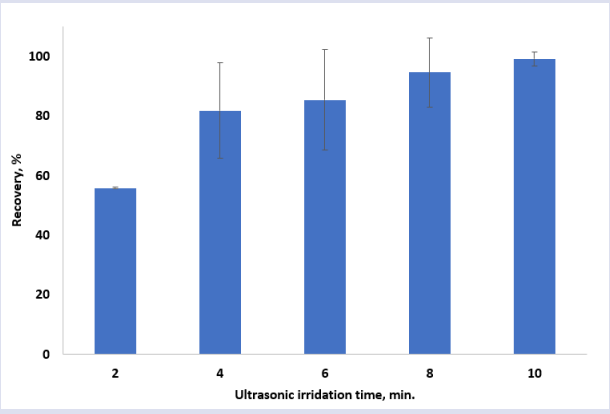

Figure 4. Effect of ultrasonic irridation time on the extraction efficiency of voriconazole (N=3).

Effect of Centrifugation Time on Extraction Efficiency of Ss-LPME

After the extraction process, centrifuge was used to separate the extraction phase and the aqueous phase. Three replicate experiments were carried out with centrifuge times varying between 2 and 10 minutes. At each stage, 2.0 mL pH 8 buffer, 200 µL decanol and 300 µL THF were added to the prepared sample solutions and the developed Ss-LPME method was applied. The highest recovery value for liquid phase microextraction of voriconazole was obtained with 8 minutes of centrifugation. It was observed that the amount of analyte passing into the supramolecular microphase was less in centrifugation processes performed for less than 8 min. Therefore, the optimum centrifuge time was determined as 8 min.

Effect of sample solution volume on extraction efficiency of Ss-LPME

For the determination of analytes with low detection limits by obtaining a high preconcentration factor, the sample volume to which a developed analytical method can be applied should be as high as possible. To determine the highest sample volume to which the developed Ss-LPME method could be applied, model solutions ranging from 15, 20, 30, 40 and 50 mL were prepared and the microextraction method was applied under optimum conditions. As shown in Figure 5, quantitative extraction efficiencies were obtained with sample solution volumes ranging from 10-40 mL. Quantitative recovery values could not be obtained in sample volumes above 40 mL. The results obtained showed that the developed method can be applied up to 40 mL sample volume. Since the final volume was 1.0 mL, a 40-fold preconcentration factor was achieved with the developed method.

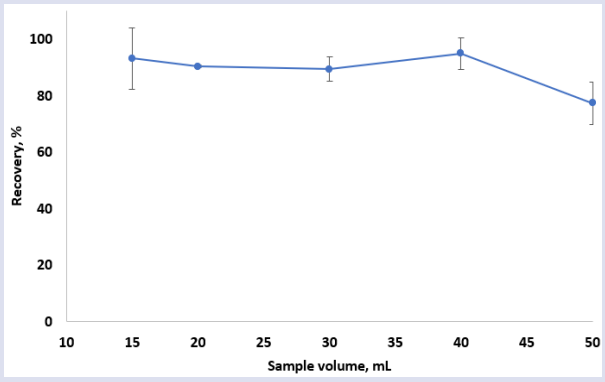

Figure 5. Effect of sample solution volume on the extraction efficiency of voriconazole (N=3).

Real Sample Analysis

The Ss-LPME method was applied to industrial wastewater and lake water samples collected from the Kayseri City region and to drug samples sold in pharmacies. Method accuracy studies were carried out with standard addition-recovery studies on these samples. The analyte concentrations specified in Table 1 were added to these samples and the developed microextraction method was applied. For this purpose, 2.0 mL of pH 8.0 buffer solution, 200 µL of 1-Decanol and 300 µL of THF were added to the analyte-added samples in 3 replicates. The samples were kept in an ultrasonic bath for 10 minutes and then centrifuged for 8 minutes. The resulting analyte phase was taken and final volume was completed to 1.0 mL with methanol. The obtained sample was vialized with appropriate procedures and measured on the HPLC. Analyzes were carried out using standard solutions prepared under the same conditions to obtain calibration curve. When the results obtained were examined, it was seen that recovery values between 100% and 108.5%. These results showed that the method we developed was applicable for these matrices.

Table 1. Standard addition-recovery and accuracy experiments for the Ss-LPME/HPLC procedure (N=3).

	Added, $mg \cdot L^{-1}$	Found, $mg \cdot L^{-1}$	Recovery, %
Waste water	0.0	ULOD[a]	-
	2.0	2.17±0.03	108.5
	4.0	4.20±0.06	105.5
Lake water	0.0	ULOD	-
	1.5	1.62±0.05	108
	3.0	3.06±0.045	102
Drug sample	0.0	2.84±0.03	-
	2.0	4.90±0.03	101
	4.0	6.82±0.03	100

ULODa: Under of the limit of detection.

Analytical Performance of Ss-LPME/HPLC Procedure

Chromatograms of voriconazole before and after the Ss-LPME method are shown in Figure 6. Calibration graph, preconcentration factor, enhancement factor, limit of detection and limit of quantification were carried out to determine the analytical performances of the Ss-LPME/HPLC procedure by using The ICH analytical method validation guidelines [19]. The developed Ss-LPME/HPLC procedure was applied to 40 mL model solutions containing increasing concentrations of voriconazole and the calibration line graph was obtained. Calibration line equation was: S=156.05C-0.2449 (S: Peak area, C: Concentration of voriconazole as $mg \cdot L^{-1}$). The enhancement factor was obtained by dividing the slope of the calibration graph obtained after applying the method by the slope of the calibration graph obtained before the method. The enhancement factor was found to be 36. The correlation coefficient (R^2) of the calibration line is 0.994. The limit of detection and limit of quantification values for the method were found to be 2.7 $\mu g \cdot L^{-1}$ and 8.8 $\mu g \cdot L^{-1}$, respectively.

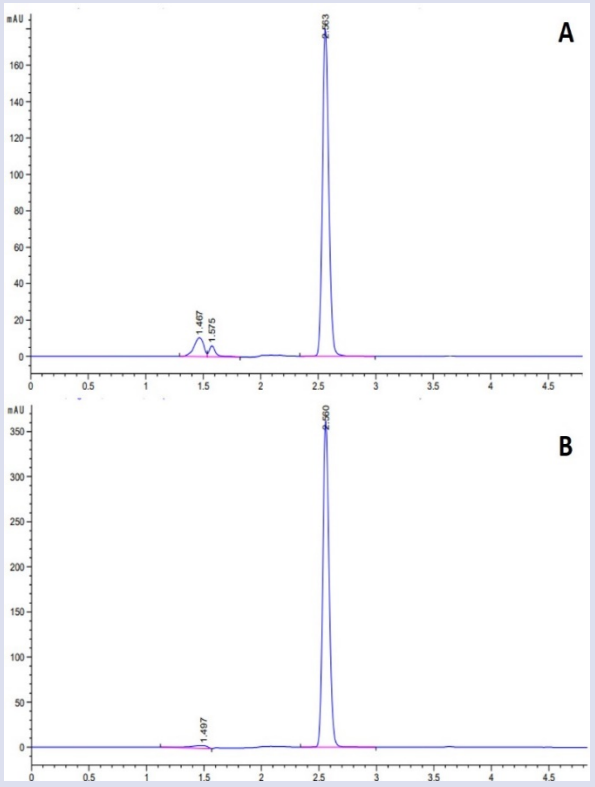

Figure 6. Chromatograms of voriconazole before (A) and after (B) the Ss-LPME method.

Conclusion

In this study, a new sample preparation method was developed to separate and enrich voriconazole in different samples by LPME based on supramolecular solvent formation. This study is the first literature study in which supramolecular solvents are used in the separation and enrichment of the active drug substance voriconazole. By combining supramolecular solvents, which are in the green solvent class, with liquid phase microextraction, which is a green analytical method, a greener analytical method has been developed compared to traditional methods. The Ss-LPME/HPLC procedure was applied to environmental water and drug samples with quantitative recoveries (>98%). Considering the above-mentioned advantages of using supramolecular solvents and liquid phase microextraction techniques, the developed Ss-LPME/HPLC procedure can be used in environmental samples and drug preparations, especially voriconazole. It has the potential to be used as a routine method in the sensitive and accurate analysis of active pharmaceutical ingredients.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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